Genetic analysis of flower color differences between a hummingbird-pollinated and a self-pollinated monkeyflower (*Mimulus*) species

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Abstract

Flower color plays an important role in pollinator discrimination and speciation. Understanding the genetic contributions to flower color differences between two closely related species, *Mimulus cardinalis* and *Mimulus parishii,* can improve understanding of how they developed different pollination syndromes and diverged from a recent common ancestor. *M. cardinalis* is hummingbird-pollinated and has large, bright red flowers while *M. parishii* is self-pollinated and has small, pale pink flowers. An F2 hybrid population between these two species was created to establish a platform for analysis of the genetic architecture controlling the differences in anthocyanin pigmentation. Statistical analysis of anthocyanin concentration distribution in the hybrid population indicated that two major loci control anthocyanin variation between *M. cardinalis* and *M. parishii*. Genetic mapping, in conjunction with the development of near-isogenic lines through serial backcrossing, located one of the major loci to a 750-kb region on chromosome four. I have also generated a near-isogenic line to isolate the other major locus. Together, my thesis work represents substantial progress towards identifying the specific genes underlying the dramatic flower color variation between a hummingbird-pollinated and a self-pollinated *Mimulus* species.

Introduction

Mimulus parishii and *Mimulus cardinalis* are two genetically similar plants with extreme variation in floral traits (Figure 1A). This study aims to discover the genes controlling the differences in flower anthocyanin concentrations between the two species. *Mimulus parishii* is a selfing plant producing small, pale pink flowers, in stark contrast to the large, bright red flowers of *M. cardinalis* (Figure 1A) and provides a platform for genetic study of flower color difference. Despite being dramatically different in color, shape and size, and never hybridizing in the wild, *M. cardinalis* and *M. parishii* are genetically very similar and can be readily crossed by manual pollination.

The genetic similarity between the two is due to the divergence of *M. parishii* and *M. cardinalis* from a common ancestor in recent evolutionary past (Beardsley et al. 2003). While *M. cardinalis* evolved to be hummingbird-pollinated, *M. parishii* evolved to be self-pollinated. This divergence in pollination mode between the species is strongly correlated with the divergence in flower color generated by distinct concentrations of two classes of pigments: anthocyanins and carotenoids. Anthocyanins are a pink pigment that are concentrated in *M. cardinalis* flowers but are virtually lacking in *M. parishii* flowers. This difference in anthocyanin production is a key factor in the reproductive isolation of these two species, as increased concentrations of anthocyanins have proven to attract hummingbirds to flowers (Schemske and Bradshaw, 1999). The lack of anthocyanin accumulation in *M. parishii* may be a consequence of a shift to self-pollination, since allocation to synthesizing these pigments would have no fitness benefit, as no pollinator is being recruited.

These characteristics of *M. cardinalis* and *M. parishii* provide a unique foundation for understanding the evolution of floral trait variation and speciation. Investigation of the genetic

architecture controlling anthocyanin pigmentation in these two species provides insight into the genetic modifications linked to the divergence in pollination mechanisms in *M. parishii* and *M. cardinalis*. This study aims to discover the genes controlling the high anthocyanin concentrations in *M. cardinalis* versus the low anthocyanin concentrations in *M. parishii* through genetic mapping of chromosomes. In order to accomplish this investigation into the genetic control of anthocyanin production, an F2 hybrid population of *M. parishii* and *M. cardinalis* was created as a platform for genetic analysis (Figure 1B).

The monkeyflower genus *Mimulus* serves as a model system for understanding developmental genetics and speciation in flowering plants. Its species have a short generation time and high fecundity, which greatly facilitate genetic analysis. Many genomic resources have developed for these species (http://mimubase.org/), and a variety of tools have been optimized for functional experimentation in monkeyflowers (Yuan et al. 2013, Ding and Yuan, 2016).

The genus *Mimulus* is also uniquely suited for genetic analysis as it harbors a great amount of phenotypic diversity among species while maintaining a high level of genetic similarity. This has led to many studies exploring the genetic and molecular basis of speciation and evolution through investigation of two closely related monkeyflower species, *Mimulus lewisii* and *Mimulus cardinalis* (Yuan et al. 2013; Bradshaw et al. 1995). One study explored the effects of flower color on pollinator preference between the two species, as *M. lewisii* is pollinated by bumblebees and *M. cardinalis* is pollinated by hummingbirds. (Bradshaw and Schemske, 2003). The study identified a locus called YELLOW UPPER (YUP), which controls the presence or absence of yellow carotenoid pigments between *M. cardinalis* and *M. lewisii*. The *M. cardinalis* YUP allele enables the production of carotenoids, while the *M. lewisii* YUP allele disables carotenoid production. These alleles determine the presence or absence of yellow

pigments in the petals and thereby determines the polllinator; the bright red flowers of *M. cardinalis* are pollinated by hummingbirds and the light pink flowers of *M. lewisii* are pollinated by bumblebees. Near-isogenic lines (NILs) were created by inserting the alternative YUP allele in the genome of *M. cardinalis* and *M. lewisii*, resulting in *M. cardinalis* plants with pink flowers containing no yellow pigments and *M. lewisii* plants with orange flowers containing yellow pigments. Exposure to pollinators revealed that hummingbirds preferentially visit flowers characterized by the presence of carotenoids and bumblebees those lacking carotenoids. Hummingbird-pollination of the *M. lewisii* NIL flowers increased 68-fold, and the bumblebee-pollination of the *M. cardinalis* NIL flowers increased 74-fold (Bradshaw and Schemske, 2003). The evolution of the two YUP alleles clearly plays a role in the two distinct pollination modes and provides insight into the mechanism of divergence of the two species, and in particular how flower trait differentiation leads to pollinator discrimination, and therefore, reproductive isolation. Thus, the genetic architecture underlying floral trait variation is useful in understanding the genetics of speciation and diversification of the >300,000 flowering plant species. It was these studies that inspired this project, because while much research has been conducted into the genetic basis of flower color difference in *M. cardinalis* and *M. lewisii* (Bradshaw and Schemske, 2003; Yuan et al. 2013; Bradshaw et al. 1995), there are many other species in the genus that provide a strong foundation for investigating the genetic causes of floral trait variation, such as *M. parishii*.

Figure 1. Flower phenotypes. (A) An inbred line of *Mimulus cardinalis* (left) was crossed with an inbred line of *Mimulus parishii* (right) to produce an F1 hybrid (middle). (B) Representative flowers from an F2 population derived from selfing a single F1 individual.